Modeling the Lag Phase and Growth Rate of *Listeria monocytogenes* in Ground Ham Containing Sodium Lactate and Sodium Diacetate at Various Storage Temperatures

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ABSTRACT: Refrigerated ready-to-eat (RTE) meats contaminated with *Listeria monocytogenes* were implicated in several listeriosis outbreaks. Lactate and diacetate have been shown to control *L. monocytogenes* in RTE meats. The objective of this study was to examine and model the effect of lactate (1.0% to 4.2%) and diacetate (0.05% to 0.2%) in ground ham on the lag phase duration (LPD, h) and growth rate (GR, log CFU/h) of *L. monocytogenes* at a range of temperatures (0 to 45 °C). A 6-strain mixture of *L. monocytogenes* was inoculated into ground ham containing lactate and diacetate, and stored at various temperatures. The LPD and GR of *L. monocytogenes* in ham as affected by lactate, diacetate, and storage temperature were analyzed and accurately represented with mathematical equations. Resulting LPD and GR equations for storage temperatures within the range of 0 to 36 °C significantly represented the experimental data with a regression coefficient of 0.97 and 0.96, respectively. Significant factors ($P < 0.05$) that affected the LPD were temperature, lactate, diacetate, and the interactions of all three, whereas only temperature and the interactions between temperature and lactate and diacetate had a significant effect on GR. At suboptimal growth temperatures (≤12 °C) the increase of lactate and diacetate concentrations, individually or in combination, extended the LPD. The effect of higher concentrations of both additives on reducing the GR was observed only at temperatures that were more suitable for growth of *L. monocytogenes*, that is, 15 to 35 °C. These data may be used to assist in determining concentrations of lactate and diacetate in cooked ham products to control the growth of *L. monocytogenes* over a wide range of temperatures during manufacturing, distribution, and storage.

Keywords: cooked ham, diacetate, growth rate, lactate, lag phase, *Listeria monocytogenes*

Introduction

Refrigerated ready-to-eat meat (RTE) products contaminated with *Listeria monocytogenes* were implicated in several listeriosis outbreaks (Centers for Disease Control and Prevention [CDC] 1998, 1999, 2002). An outbreak in 1998 in the United States caused by a single strain of *L. monocytogenes* resulting in 40 illnesses in 10 states was linked to the consumption of contaminated hot dogs (CDC 1998). An outbreak in 2002 in the United States resulting in 7 deaths, 3 stillbirths or miscarriages, and a recall of 12.4 million kg of implicated products was linked to turkey deli meat products (CDC 2002). The prevalence of *L. monocytogenes* in sliced luncheon meat ranged from 4.2% to 8.0% in 2300 samples collected from federally inspected establishments between 1990 and 1999 in the US (Levine and others 2001). Gombas and others (2003) reported a prevalence of *L. monocytogenes* of 0.89% (82/9199) in RTE luncheon meats collected from grocery stores in California and Maryland in the United States between 2000 and 2001. In a study examining the prevalence of *L. monocytogenes* in packages of frankfurters obtained from several commercial manufacturers over a 2-y period, Wallace and others (2003) reported that 1.6% (532/32800) of the packages were positive for *L. monocytogenes*. The possible contamination of *L. monocytogenes* in RTE meat, beef and chicken products such as corned beef, pastrami, and frankfurters, is one of the main reasons for Class I type food recalls in the United States (FSIS 2005). A survey conducted between 1997 and 1998 on the Belgian retail market showed that 4.9% (167/3405) of cooked meat products were contaminated with *L. monocytogenes*, and a higher incidence rate was observed for whole cooked meat products after slicing (6.65%) than before slicing (1.56%) (Uyttendaele and others 1999).

A risk assessment reported that deli meats had the highest estimated per annum risk of illness and death from *L. monocytogenes* among 20 RTE food categories (FDA/USDA/CDC 2003). While the heat processing used in the manufacturing of presliced, prepackaged RTE meat products is sufficient to eliminate *L. monocytogenes*, recontamination may occur during postprocessing steps such as slicing, packaging, or handling at manufacturing, retail, or the consumer level. To reduce the health hazards of *L. monocytogenes*, food additives or antimicrobials such as nisin, organic acids, benzoate, sorbate (Samelis and other 2001, 2005), lactate, acetate (Nerbrink and others 1999; Mbandi and Shelef 2001; Stekelenburg and Kant-Muermans 2001; Stekelenburg 2003; Samelis and others 2005; Luchansky and others 2006a), acidic calcium sulfate, and lauric arginate (Luchansky and others 2006b), protective bacterial cultures (Bredholt and others 1999), or bacteriophage (FDA 2006) have

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been evaluated for their ability to control *L. monocytogenes* in RTE meats. Lactate and diacetate salts have been extensively evaluated as antimicrobial additives and are affirmed by the U.S. FDA as generally recognized as safe (GRAS) and can be used as a direct human food ingredient (21 CFR 184.1754 and 184.1768). Lactate at levels not exceeding 4.8% by weight of total formulation is permitted in various meat and poultry products to inhibit microbial growth, and diacetate levels not exceeding 0.25% are permitted by regulation (9 CFR Part 424).

The effect of lactate and diacetate on *L. monocytogenes* in RTE meat and poultry products has been reported. Blom and others (1997) reported that a combination of 2.5% lactate and 0.25% diacetate controlled the growth of *L. monocytogenes* in vacuum-packaged sliced cooked ham at 4 °C, but they were less effective when the product was stored at 9 °C. Mbandi and Shelef (2001) reported that a combination of 2.5% lactate and 0.25% diacetate prevented the growth of *L. monocytogenes* in beef sausage stored at 4 °C. A model predicting the growth rate (GR) of *L. monocytogenes* in cured RTE processed meat as affected by the levels of sodium chloride, sodium diacetate, potassium lactate, and moisture in the products at 4 °C was reported by Seman and others (2002). The model showed that increasing amounts of potassium lactate and sodium diacetate in RTE meat significantly reduced the GR of *L. monocytogenes* correspondingly at 4 °C. Another study modeling the growth boundary of *L. monocytogenes* in RTE cooked meat products as a function of salt (0.8% to 3.6%), sodium diacetate (0% to 0.2%), potassium lactate syrup (0.25% to 9.25%), and finished product moisture content (45.5% to 83.5%) was also conducted at 4 °C (Legan and others 2004). Barmpalia and others (2005) examined the effect of sodium lactate (1.8%) and sodium diacetate (0.125% and 0.25%) on the GR of *L. monocytogenes* in pork bologna stored at 4 and 10 °C, and reported that the lowest GR occurred in samples containing 1.8% sodium lactate and 0.25% sodium diacetate. Glass and others (2002) also reported that sodium lactate (3.4%) and sodium diacetate (0.1%) effectively inhibited the growth of *L. monocytogenes* in bratwurst at 3 and 7 °C. Lactate and diacetate were also examined by Uhart and others (2004) for use as a dipping solution for beef frankfurters. They reported a reduction of about 1.0 log CFU/g for a 4-strain mixture of *L. monocytogenes* during storage at 4 °C for 2 wk.

Studies examining the effects of lactate and diacetate on *L. monocytogenes* have been conducted mainly at refrigerated and mild abuse temperatures, for example, 8 to 12 °C. However, RTE meat products may be exposed to higher temperatures during manufacturing (for example, cooling after heat processes), distribution, and storage at retail and consumer levels. In this regard, there is little information about the effects of lactate and diacetate on *L. monocytogenes* in RTE meat products over a wider range of storage temperatures. Therefore, the objective of this study was to examine and model the effect of sodium lactate (1.0% to 4.2%) and sodium diacetate (0.05% to 0.2%) on *L. monocytogenes* in ground ham at the temperature range of 0 to 45 °C.

Materials and Methods

Experimental design

A central composite design (SAS 9.1 for Windows, SAS Institute Inc., Cary, N.C., U.S.A.) was used to select treatments of various combinations of concentrations of lactate (1.0% to 4.2%), and diacetate (0.05% to 0.2%) in ham, and storage temperature (0 to 45 °C). The design selected 23 runs (runs 1 to 23, Table 1) for which 9 runs were the repeats of the central point. The design represented 15 treatments consisting of various combinations of the additives and storage temperature. In addition, 4 treatments were added to examine higher storage temperatures and higher additive concentrations (runs 24 to 27, Table 1). Therefore, a total of 19 treatments were examined in this study. Sample preparation, inoculation, and storage are described subsequently. The experiment was performed twice (2 trials) with duplicate samples at each sampling interval for each of the 2 trials.

Table 1 — Experimental runs selected by the central composite design for storage temperatures 0 to 45 °C, sodium lactate 1.0% to 4.2% and sodium diacetate 0.05% to 0.2%

Run	Storage temperature (°C)	Sodium lactate (%)	Sodium diacetate (%)
1	9.1	1.65	0.080
2	9.1	3.55	0.080
3	9.1	1.65	0.170
4	9.1	3.55	0.170
5	35.9	1.65	0.080
6	35.9	3.55	0.080
7	35.9	1.65	0.170
8	35.9	3.55	0.170
9	0.0	2.60	0.125
10	45.0	2.60	0.125
11	22.5	2.60	0.050
12	22.5	2.60	0.200
13	22.5	1.00	0.125
14	22.5	4.20	0.125
15	22.5	2.60	0.125
16	22.5	2.60	0.125
17	22.5	2.60	0.125
18	22.5	2.60	0.125
19	22.5	2.60	0.125
20	22.5	2.60	0.125
21	22.5	2.60	0.125
22	22.5	2.60	0.125
23	22.5	2.60	0.125
24	45.0	4.20	0.200
25	45.0	2.60	0.200
26	45.0	4.20	0.125
27	22.5	4.20	0.200

L. monocytogenes strains and inoculum preparation

The 6 strains of *L. monocytogenes* used in this study were obtained from the culture collection of the Microbial Food Safety Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Dept. of Agriculture. The strains (source of isolate, serotype) were F6854 (turkey frank, 1/2a), MFS−2 (environmental isolate from a pork processing plant, 1/2a), H7776 (frankfurter, 4b), JBL2365 (chocolate milk, 4b), F2365 (Hispanic-style cheese, 4b), and 101 M (ground beef, 4b) (Porto and others 2003). Each strain was transferred from a stock culture held at −80 °C into 10-mL Brain Heart Infusion broth (BHI, Becton, Dickinson and Co., Sparks, Md., U.S.A.) and incubated at 37 °C for 4 h. A loopful of cell suspension of each strain was then separately transferred to fresh 10-mL BHI broth and incubated at 37 °C for 24 h. An equal volume of cell suspension from each strain was mixed together, and the mixture was further diluted with sterile 0.1% peptone water to achieve a population of about 10^5 CFU/ml for use as an inoculum.

Ground ham preparation

Cooked ham samples were used in this study and kindly provided by a commercial processor of pork products. The ham was formulated with common ingredients (salt, sugar, sodium phosphate, sodium erythorbate, and sodium nitrite) and was kept frozen (−80 °C) until experimentation. To ensure that the exact amounts

of both additives were present in the experimental ham, sodium lactate and sodium diacetate were not added as ingredients until after the ham was ground before the experiment. To prepare ham samples for inoculation, the required amounts of frozen ham were tempered overnight at 4 °C, and then cut into 2-mm-thick slices using a Globe 3500 meat slicer (Globe Food Equipment Co., Dayton, Ohio, U.S.A.). Ham slices were then double ground through a 3-mm plate using a Univex 8912 meat grinder (Univex Inc., Salem, N.H., U.S.A.). Ground ham (100 g) was placed in 500-mL capacity Prime Source 3-mil high barrier vacuum pouches (Koch Supplies Inc., North Kansas City, Mo., U.S.A.) and vacuum-sealed to −980 mbar using a Multivac vacuum sealer (Multivac Inc., Kansas, Mo., U.S.A.). Fifteen bags were vacuum-sealed in MIL-PRF-131J foil bags (Ludlow Coated Products Inc., Columbus, Ga., U.S.A.) and stored at −80 °C. The ground ham was irradiated to sterility with Cobalt-60 at 4.2 kGy (Food Technology Service Inc., Mulberry, Fla., U.S.A.) to eliminate background microflora. Packages of irradiated ham were kept at −80 °C until use. The irradiated ground ham was analyzed for proximate analysis (moisture 71.1%, protein 20.27%, fat 5.9%, salt 1.88%, total phosphate 0.238%, nitrite < 1 ppm, and erythorbate < 1 mg/100 g) by Meat Industry Laboratories Inc. (Chicago, Ill., U.S.A.).

Sample inoculation and storage

Packages of frozen ground ham were thawed at room temperature and supplemented with the desired amounts of sodium lactate and sodium diacetate as shown in Table 1. Sodium lactate (Purasal Power S 96, Purac America, Lincolnshire, Ill., U.S.A.) and sodium diacetate (Spectrum Chemical Manufacturing Corp., New Brunswick, N.J., U.S.A.) were dissolved in 5-mL sterile deionized water before addition to bags containing 100 g ground ham. The packages were hand-massaged for 30 s, and then stomached for 2 min. Five gram portions of ham were packed into 100-mL stomacher bags (Spiral Biotech Inc., Norwood, Mass., U.S.A.). The bags were stored at the selected storage temperatures for 30 min to equilibrate the sample prior to *L. monocytogenes* inoculation. One-tenth of 1 mL of the 10^5 CFU/ml 6-strain *L. monocytogenes* cocktail was added into each bag and mixed thoroughly by hand. The bags were vacuum-sealed to −980 mbar using a Multivac A300 vacuum sealer and stored at the selected storage temperatures (Table 1). The initial pH of ham before inoculation was measured with a Corning pH meter model 430 fitted with a Corning "3 in 1" combo electrode (Corning Inc., New York, N.Y., U.S.A.), and the initial water activity was measured with an AquaLab model CX2 water activity meter (Decagon Devices, Inc., Pullman, Wash., U.S.A.). Duplicate samples were used in pH and water activity measurements. The initial pHs of samples were pH 6.0 to 6.3 and a_w 0.96 to 0.98.

Enumeration of *L. monocytogenes*

Levels of *L. monocytogenes* in ham samples were enumerated at selected time intervals that allowed an accurate estimation of lag phase duration (LPD) for *L. monocytogenes* in ham at each storage temperature. At each sampling time, 2 bags were aseptically opened, and an equal weight of sterile 0.1% peptone water was added into the bags. The bags were stomached for 1 min in a Bag-Mixer 400 Stomacher (Interscience Inc., St. Nom, France). Additional dilutions, if needed, were prepared with sterile 0.1% peptone water. Duplicate dilutions (50 μL) were spread-plated onto each of 2 modified Oxford agar plates (MOX, Oxoid Ltd., Hampshire, U.K.). Plates were incubated at 37 °C for 48 h and black colonies surrounded by black precipitation that were identical to those of a *L. monocytogenes* stock culture on MOX plates were counted. If needed, colonies recovered from samples were confirmed using API *Listeria* test strips (BioMerieux, Marcy l'Etoile, France). Regularly, samples were also plated on tryptic soy agar (Becton, Dickinson and Co., Sparks, Md., U.S.A.) to compare the total counts and *L. monocytogenes* counts for possible contamination.

Growth curve-fitting and model development

The DMFit software (http://www.ifr.ac.uk/Safety/DMfit/default.html) based on the work of Baranyi and Roberts (1994) was used to fit the growth curves of *L. monocytogenes* in ham samples to estimate the lag phase duration (LPD, h), growth rate (GR, $\log_{10}$ CFU/h), and maximum population density (MPD, log CFU/g). Since MPD did not significantly differ among the various treatments of lactate, diacetate, and storage temperature, MPD was not included in the modeling. The LPD and GR were analyzed by the General Linear Model of SAS 9.1 for analysis of variance. The LPD and GR were fitted as a function of the concentrations of sodium lactate and sodium diacetate, storage temperature, and interactions terms, using the following quadratic response equation:

$$\text{LPD or GR} = \alpha + \beta1(T) + \beta2(L) + \beta3(D) + \beta4(T * L) + \beta5(T * D) + \beta6(L * D) + \beta7(T)^2 + \beta8(L)^2 + \beta9(D)^2$$

where T is the storage temperature (°C), L is the concentration of sodium lactate (%), D is the concentration sodium diacetate, α is the intercept, and $\beta1$ to $\beta9$ are estimated coefficients for each parameter and their interactions.

The regression of LPD or GR on lactate, diacetate, and storage temperature had the best fit when data from only 0 to 35.9 °C were included in the regression analysis. Since no growth of *L. monocytogenes* occurred in ham samples stored at 45 °C, the regression analysis included only LPD or GR obtained from experiments with storage temperatures of 0 to 35.9 °C.

Evaluation of model performance

The performance of the LPD and GR equations in predicting LPD and GR of *L. monocytogenes* in ham was validated by independent experiments. Samples of ground ham containing 1.5% or 3.0% sodium lactate and 0.10% or 0.15% sodium diacetate were inoculated with *L. monocytogenes*, as described previously, and stored at 4, 8, 12, 10, 16, and 25 °C. The LPD and GR of *L. monocytogenes* at each storage temperature were obtained from experimental data using DMFit. The LPD and GR obtained from the experimental values were compared to predicted values calculated from LPD and GR secondary models described previously using a bias factor ($B_f = 10^{(\sum \log(\text{LPDorGRpredicted/LPDorGRobserved})/n)}$) and an accuracy factor ($A_f = 10^{(\sum |\log(\text{LPD/GRpredicted/LPDorGRobserved})|/n)}$). The B_f indicates that, on average, the prediction is higher (>1.0) or lower (<1.0) than the observed value, while the A_f indicates the average closeness of the prediction to the observation (Ross 1996).

Results and Discussion

LPD and GR of *L. monocytogenes* in ham

The LPD, GR, and MPD for *L. monocytogenes* in ground ham containing various concentrations of lactate and diacetate at temperatures between 0 and 45 °C are shown in Table 2. LPD and MPD are not reported for *L. monocytogenes* when growth was not observed. In ham that supported growth, the MPD reached approximately 7.0 to 8.0 log CFU/g. Regardless of the concentrations of lactate (1.0% to 4.2%) or diacetate (0.05% to 0.2%) in ham used in this study, *L. monocytogenes* grew at 9.1, 22.5, or 35.9 °C. Lactate and diacetate at suboptimal growth temperatures (0 and 45 °C)

Table 2—Means of LPD, GR, and MPD (standard deviation) of *L. monocytogenes* in irradiated ground ham containing various levels of sodium lactate and sodium diacetate stored at selected temperatures

Temperature (°C)	Lactate (%)	Diacetate (%)	*n*	LPD (h)	GR (log CFU/h)	MPD (log CFU/g)
0	2.6	0.125	2	—	−0.0004 (0.0002)	—
9.1	1.65	0.08	2	80.6 (9.6)	0.0205 (0.0007)	8.5 (0.1)
9.1	1.65	0.17	2	174.2 (14.2)	0.0175 (0.0007)	8.5 (0.1)
9.1	3.55	0.08	2	171.7 (20.7)	0.0130 (0.0028)	7.6 (0.3)
9.1	3.55	0.17	2	203.2 (4.0)	0.0160 (0.0014)	7.1 (0.2)
22.5	1.0	0.125	2	10.6 (6.1)	0.1205 (0.0078)	7.1 (0.7)
22.5	2.6	0.05	2	9.2 (0.7)	0.0915 (0.0148)	7.0 (0.1)
22.5	2.6	0.125	10	16.1 (8.9)	0.0671 (0.0120)	8.5 (0.2)
22.5	2.6	0.2	2	41.2 (34.3)	0.0820 (0.0000)	6.7 (0.4)
22.5	4.2	0.125	2	50.4 (26.1)	0.0465 (0.0191)	7.4 (0.6)
22.5	4.2	0.2	2	70.1 (3.9)	0.0435 (0.0134)	7.8 (0.2)
35.9	1.65	0.08	2	3.9 (0.6)	0.2425 (0.0064)	7.9 (0.6)
35.9	1.65	0.17	2	2.7 (1.9)	0.1525 (0.0134)	7.2 (0.1)
35.9	3.55	0.08	2	16.5 (2.4)	0.1760 (0.0042)	7.9 (0.1)
35.9	3.55	0.17	2	21.5 (22.3)	0.0470 (0.0495)	7.4 (0.2)
45	2.6	0.125	2	—	−0.0800 (0.0382)	—
45	2.6	0.2	2	—	−0.0830 (0.0085)	—
45	4.2	0.125	2	—	−0.0335 (0.0078)	—
45	4.2	0.2	2	—	−0.1285 (0.0007)	—

had an inactivating effect on *L. monocytogenes* in ham. The greatest inactivation rate (GR = −0.1285 log CFU/h) was in ham containing the highest amounts of lactate (4.2%) and diacetate (0.2%) and stored at 45 °C. The inactivation rate of *L. monocytogenes* in ham containing 2.6% lactate and 0.125% diacetate was greater at 45 °C (−0.080 log CFU/h) than at 0 °C (−0.0004 log CFU/h). At conditions permitting *L. monocytogenes* growth, the LPD ranged from approximately 3 h in ham containing 1.65% lactate and 0.17% diacetate stored at 35.9 °C to approximately 203 h in ham containing 3.55% lactate and 0.17% diacetate stored at 9.1 °C. The highest GR (0.2425 log CFU/h) was observed in ham containing 1.65% lactate and 0.08% diacetate stored at 35.9 °C. The temperatures that supported the growth or survival of *L. monocytogenes* in ham observed in this study agree with previous reports of the minimal, optimal, and maximum growth temperatures (approximately −0.4, 37, and 45 °C) for *L. monocytogenes*, respectively (ICMSF 1996).

LPD response surface model

The mathematical equation that describes the observed LPD of *L. monocytogenes* in ham as a function of the concentrations of lactate (L) and diacetate (D), and storage temperatures (T) of 0 to 35.9 °C is:

$$\text{LPD (h)} = 79.97 - 13.17\text{T} - 35.76\text{L} + 1059.73\text{D} - 1.03\text{TL} - 28.55\text{TD} - 176.53\text{LD} + 0.31\text{T}^2 + 4.93\text{L}^2 + 1300.49\text{D}^2$$

[Correction added after online publication 7 Aug 2007: in the preceding equation, 79.9713.17T was corrected to 79.97 − 13.17T]

The equation was significant ($P < 0.001$) in describing the LPD obtained from the experiment with a regression coefficient (R^2) of 0.97. The significance levels of lactate, diacetate, and storage temperature on affecting (increasing or decreasing) the LPD are shown in Table 3. Both the concentration of lactate and diacetate in ham and the storage temperature significantly ($P < 0.05$) affected the LPD. The interactions between temperature and lactate, temperature and diacetate, and lactate and diacetate also significantly ($P < 0.05$) affected the LPD. The effects of lactate and diacetate on the LPD at storage temperatures of 4, 8, 12, 15, 25, and 35 °C, which are relevant to storage, abuse, and processing temperatures, are shown in response surface plots (Figure 1A to 1F) derived from the LPD equation. The plots showed that the decrease of storage temperature increased the LPD of *L. monocytogenes*. At storage temperatures of 4, 8, 12, and 15 °C, an increase of lactate or diacetate increased the LPD of *L. monocytogenes* in a linear fashion (Figure 1A to 1D). At each of these 4 storage temperatures, *L. monocytogenes* had the longest LPD in ham containing the highest concentrations of lactate (4.2%) and diacetate (0.2%).

At storage temperatures of 25 and 35 °C, an increase of lactate and diacetate did not proportionally increase the LPD of *L. monocytogenes* (Figure 1E and 1F). Specifically, at 25 °C in ham containing the lowest concentration of acetate (0.05%) or lactate (1.0%), the increase of lactate or diacetate had no effect on increasing LPD until the lactate concentrations were higher than 2.5% or when diacetate concentrations were higher than 0.11% (Figure 1E). At lactate concentrations greater than 2.5%, the effect of increased lactate concentrations in increasing LPD was more significant at lower diacetate concentrations. Thus, at higher diacetate concentrations the increase of lactate did not increase the LPD significantly. Similarly, the increase of lactate concentrations in increasing the LPD was more significant in ham containing lower concentrations of diacetate (Figure 1E). At 35 °C, higher concentrations of lactate increased LPD only at diacetate concentrations that were less than 0.125%. An increase in levels of diacetate increased LPD only at lactate levels less than 2.6% (Figure 1F). The response surface plots indicate that increases in both lactate and diacetate concentrations delaying the growth at storage temperatures ≤ 15 °C.

The effect of increasing lactate or diacetate concentrations on delaying growth of *L. monocytogenes* at lower temperatures has also been reported. Seman and others (2002) found that an increase in lactate and diacetate concentrations significantly reduced the

Table 3—Coefficients and significance levels for each parameter in the LPD and GR equations

	LPD equation		GR equation	
Parameter	Coefficient	Pr > |t|	Coefficient	Pr > |t|
Intercept	79.97	0.0523	−0.0146	0.7327
T	−13.17	<0.0001	0.0098	<0.0001
L	35.76	0.0369	−0.0206	0.2463
D	1059.73	0.0056	−0.2220	0.5584
TL	−1.03	0.0006	−0.0013	<0.0001
TD	−28.55	<0.0001	−0.0392	<0.0001
LD	−176.53	0.0138	0.0143	0.8417
T^2	0.31	<0.0001	0.0001	0.0002
L^2	4.93	0.088	0.0053	0.0815
D^2	1300.49	0.314	2.9529	0.0335

growth of *L. monocytogenes* at 4 °C. Bedie and others (2001) reported that sodium lactate at 3% or sodium diacetate at 0.25% was inhibitory to *L. monocytogenes* in frankfurters stored at 4 °C for 70 or 35 to 50 d, respectively. While the concentrations of lactate or diacetate were increased to 6% or 0.5%, respectively, the inhibitory effect was extended to 120 d. Samelis and others (2002) reported that a combination of 1.8% sodium lactate and 0.25% sodium diacetate inhibited growth of *L. monocytogenes* in frankfurters for 120 d at 4 °C compared to 35 to 50 d when sodium lactate was used alone. Stekelenburg (2003) reported that growth of *L. monocytogenes* in frankfurters was suppressed significantly at 4 °C during a 29-d storage by the combination of 2% to 3% of a 65% potassium lactate solution and 4% sodium diacetate than the use of just 3% potassium lactate or 0.1% potassium diacetate.

The effect of lactate and diacetate on suppressing *L. monocytogenes* growth increased as the levels of both additives increased during refrigerated storage or mild abuse storage temperatures. However, the present study showed that, at higher storage temperatures, an increase in lactate or diacetate did not increase the LPD. This implies that the increasing growth suppression effect of higher lactate and diacetate levels may have a threshold effect only at temperatures that are suboptimal for the growth of *L. monocytogenes*. At temperatures close to the optimal growth boundary of *L. monocytogenes*, the effect of higher concentrations of both additives in suppressing growth is diminished.

GR response surface model

The regression equation describing the GR of *L. monocytogenes* in ham containing lactate and diacetate, and stored at 0 to 35.9 °C is:

$$\begin{aligned}\mathrm{GR(log\ CFU/h)} &= -0.0146 + 0.0098\mathrm{T} - 0.0206\mathrm{L} - 0.2220\mathrm{D} - 0.0013\mathrm{TL} \\ &\quad - 0.0392\mathrm{TD} + 0.0143\mathrm{LD} + 0.0001\mathrm{T}^2 + 0.0053\mathrm{L}^2 + 2.9529\mathrm{D}^2\end{aligned}$$

The equation is significant ($P < 0.001$) in describing the observed GR from the experiments with a regression coefficient of 0.96. The significance levels of lactate, diacetate, and storage temperature affecting the GR are shown in Table 3. The storage temperature and the interaction between temperature and lactate or diacetate had significant effects on GR. The latter indicated that the effect of both additives on the GR of *L. monocytogenes* was influenced by the storage temperature. Plots of response surfaces showing GR as a function of lactate and diacetate concentrations at 4, 8, 12, 15, 25, and 35 °C are shown in Figure 2A to 2F. As expected, an increase in storage temperature increased the GR of *L. monocytogenes* in ham. The increases in lactate and diacetate levels did not exhibit a consistent effect on GR. At lower temperatures, for example, 4 and 8 °C, an increase in diacetate concentration increased GR, while an increase in lactate concentration had little effect on GR (Figure 2A and 2B). At 12 and

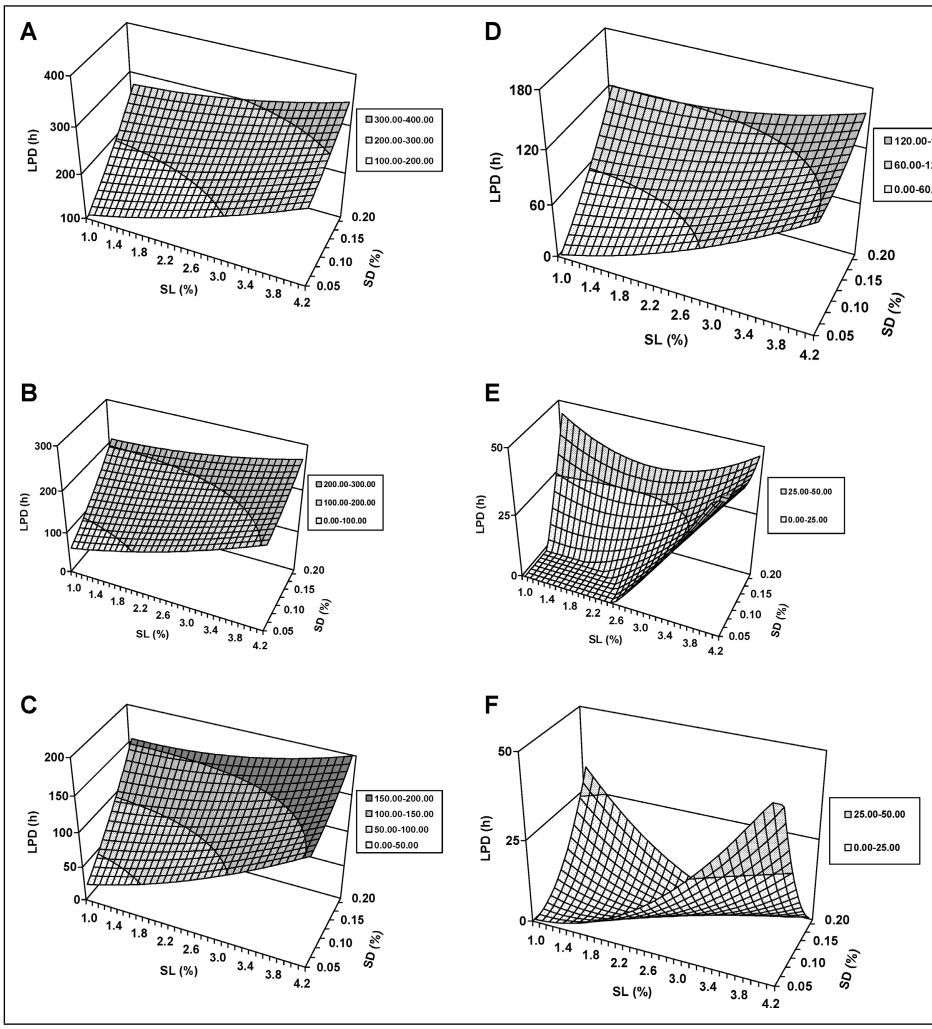

Figure 1—Plots of response surfaces of the LPD equation describing the LPD of *L. monocytogenes* in ham containing 1.0% to 4.2% sodium lactate and 0.05% to 0.2% sodium diacetate stored at 4 (A), 8 (B), 12 (C), 15 (D), 25 (E), and 35 °C (F)

15 °C, an increase in lactate concentration decreased GR, whereas diacetate had less of the effect (Figure 2C and 2D). At 25 and 35 °C, an increase in concentrations of both lactate and diacetate decreased GR. Results showed that the effect of increasing lactate or diacetate concentration in reducing GR occurred only at temperatures that were relatively more favorable for *L. monocytogenes* growth.

Seman and others (2002) reported a model to predict GR of *L. monocytogenes* in cured RTE processed meat at 4 °C as a function of the levels of sodium chloride, sodium diacetate, potassium lactate, and moisture. The model showed that increased amounts of potassium lactate and sodium diacetate in RTE meat significantly reduced the GR of *L. monocytogenes*. The model predictions appeared to be reasonable and expected. However, it is possible that some *L. monocytogenes* strains used in the present study adapted to high concentrations of lactate and diacetate during the extended lag phase at refrigerated temperatures and were able to grow rapidly once entering exponential growth phase.

Model validation

The LPD and GR of *L. monocytogenes* in irradiated ground ham calculated from the observed data for the validation samples were compared to predictions from the secondary models to determine indices of the model's performance (Table 4). In evaluating model

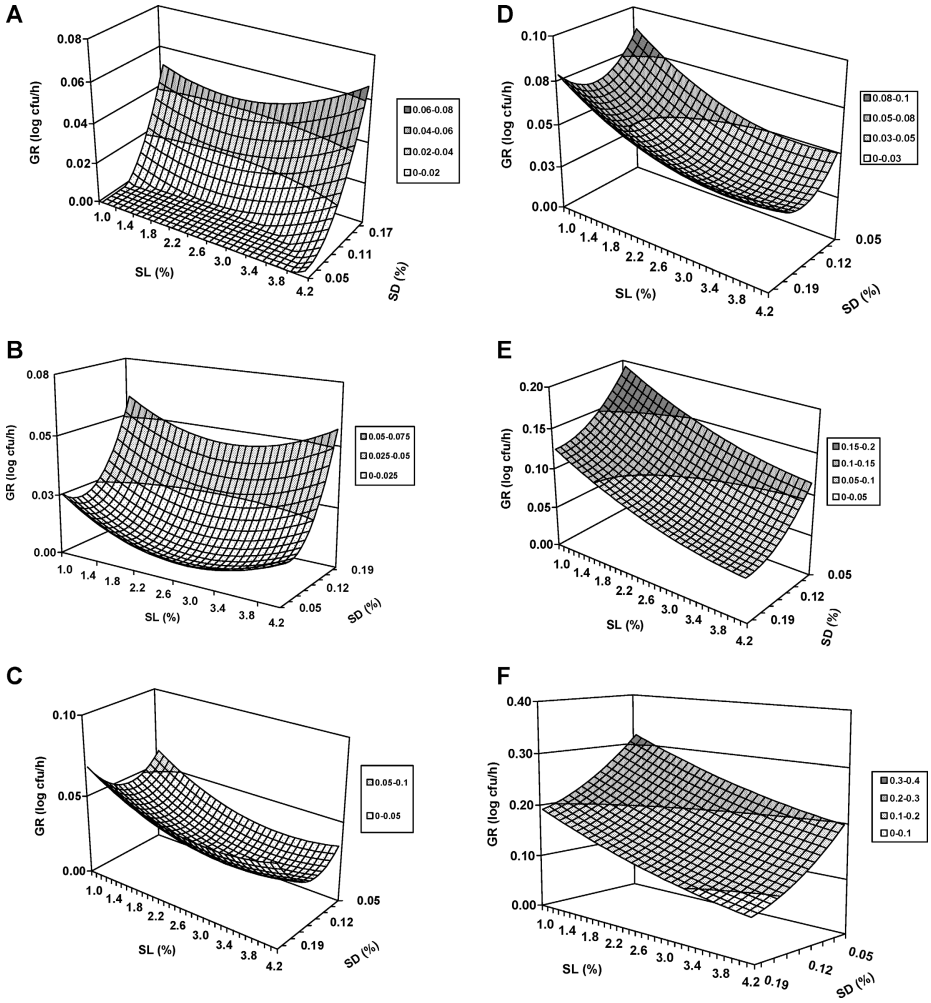

Figure 2—Plots of response surfaces of the GR equation describing the GR of *L. monocytogenes* in ham containing 1.0% to 4.2% sodium lactate and 0.05% to 0.2% sodium diacetate stored at 4 (A), 8 (B), 12 (C), 15 (D), 25 (E), and 35 °C (F)

Table 4—Bias factor (B_f) and accuracy factor (A_f) of the LPD and GR equations in predicting LPD and GR of *L. monocytogenes* in ham containing lactate and diacetate stored at various temperatures

			LPD (h)		GR (log CFU/h)	
Temperature (°C)	Lactate (%)	Diacetate (%)	Obs.	Pred.	Obs.	Pred.
4	3.0	0.10	296.4	226.1	−0.0027	−0.0076
8	3.0	0.10	168.4	164.6	0.006	0.0052
8	1.5	0.15	134.7	161.1	0.0101	0.0249
10	1.5	0.15	130.8	134.2	0.0312	0.0325
12	3.0	0.10	103.2	112.9	0.0187	0.0211
12	1.5	0.15	102.7	109.9	0.0386	0.0408
16	1.5	0.15	27.7	68.6	0.0504	0.0599
25	1.5	0.15	8.1	12.0	0.1032	0.1145
			B_f	1.19	B_f	1.21
			A_f	1.28	A_f	1.17

performance, an A_f of 1.0 indicates that the model, on average, produces a perfect prediction (predicted = observed). Model performance (prediction) is also considered acceptable if A_f values increase or decrease 0.15 units for each modeled variable (Ross and others 2000). In the present study, there are 3 model variables (lactate, diacetate, and temperature); therefore, an acceptable A_f value would be between 0.55 and 1.45. Overall, although the predicted LPD were 28% ($A_f = 1.28$) higher ($B_f = 1.19$) than the observed values, the LPD model is considered acceptable. Similarly, although the predicted GR was higher ($B_f = 1.17$) than the observed value by 21% ($A_f = 1.21$), the GR model is considered acceptable. Hence, the combination of LPD and GR models of *L. monocytogenes* in ham may be reasonably used to estimate the growth of *L. monocytogenes* in ham containing lactate and diacetate at the temperature range of 0 to 36 °C for the type of ham product studies herein. The objective of this study was to develop models to cover a wide range of concentrations of sodium lactate and sodium diacetate and a wide range of temperature that cooked ham products may be exposed to. In order to cover the wide range of the 3 parameters and make the experiment manageable, treatments examined were obtained from a central composite design and a few additional ones. The resulted models were validated with a limited set of data and proved to be acceptable. However, it is recognized that additional validations will further assist in defining the model's representativeness and robustness. While these models may be applicable to the wide range of parameters examined in this study, they can be further improved with data obtained from the concentrations of lactate and diacetate and the temperatures that are of interest in cooked ham products during manufacturing and distribution. These data may include those from treatments containing concentrations of lactate and diacetate that are more sensory acceptable and temperatures that are more relevant to the conditions during the distribution and storage of cooked ham production.

Conclusions

Lactate and diacetate are increasingly being added into various cooked meat products to control *L. monocytogenes* outgrowth during shelf life. Levels of both additives vary among products and are often determined from studies specifically conducted for a particular product formulated with selected levels of both additives, individually or combination, and for few storage temperatures, mainly refrigerated temperatures. This study elucidated the effect of a wider range of storage temperature and lactate and diacetate levels on the LPD and GR of *L. monocytogenes* in cured irradiated ground ham. Results from this study not only confirm that the addition of lactate and diacetate delays growth *L. monocytogenes*, particularly at lower storage temperatures, but also provide new information regarding the interactions of lactate, diacetate and storage temperature on *L. monocytogenes*. The resulting LPD and GR models may be used together as a generic model to predict the behavior of *L. monocytogenes* in cooked ham products to facilitate defining the concentrations of lactate and diacetate in ham formulation to achieve the desired product safety, with consideration of temperatures that the product may be exposed to during manufacturing, distribution, and consumer storage.

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